

OBSERVATIONS ON CLONAL CULTURES OF *EUGLYPHA ACANTHOPHORA* AND *EUGLYPHA* *STRIGOSA* (TESTACEA: PROTOZOA)

By R. H. HEDLEY, C. G. OGDEN & JUNE I. KRAFFT

INTRODUCTION

MANY testate amoebae are known only from their original descriptions, based on observations made at the magnification limits of the optical microscope. The introduction of scanning-electron microscopy for surface ultrastructure examination now allows more accurate descriptions to be made of these animals. The present account describes the shell morphology and biology of two species of *Euglypha* Dujardin, 1841, namely *Euglypha acanthophora* (Ehrenberg, 1841) and *Euglypha strigosa* (Ehrenberg, 1871), both of which have been recently isolated from samples of sewage sludge and moss respectively, collected in southern England. This is the third paper in a series dealing with testate amoebae. The previous papers (Hedley and Ogden, 1973, 1974) are devoted to the biology and fine structure of *Euglypha rotunda* and *Trinema lineare* respectively. Fine structure observations on the cytoplasm of *E. acanthophora* and *E. strigosa* will be the subject of a separate publication.

The genus *Euglypha* belongs to the family Euglyphidae – the classification adopted here is that proposed by Loeblich and Tappan (1961) :

Class	<i>RHIZOPODEA</i>	Von Siebold, 1845
Subclass	<i>FILOSIA</i>	Leidy, 1879
Order	<i>GROMIDA</i>	Claparède and Lachmann, 1859
Superfamily	EUGLYPHACEA	Loeblich and Tappan, 1961
Family	EUGLYPHIDAE	Wallich, 1864
		test hyaline, symmetrical, elongate, composed of rounded siliceous scales, aperture rounded or elongate ; one nucleus.

Previous work – biology

Euglypha acanthophora. Most of the early biological observations of *Euglypha* were made on this species. These reports (Leidy, 1877 ; Gruber, 1881 ; Blochmann, 1887 ; Schewiakoff, 1888 ; Carter, 1889 ; Penard, 1890 ; Reukauf, 1912 ; Popoff, 1912) dealt mainly with nuclear division and reproduction by binary fission. Carter (1865, 1889), Reukauf (1912) and Popoff (1912) also described another process of division in which many individuals were produced at one time, but all the stages of this division were not seen. A report by Jones (1929), made from observations on an

old protozoan culture, described a flagellate stage for *E. acanthophora*. This has not been confirmed and it is possible that the cultures were contaminated.

Encystation was observed by Penard (1890, 1902), Leidy (1879), Popoff (1912) and Reukauf (1912). Leidy (1879) described the cyst as being enclosed in two distinct envelopes, and Popoff (1912) illustrated the various stages in the process of encystation. When two individuals were observed joining to form a single large specimen and immediately formed a cyst, Reukauf (1912) suggested that this was a copulation cyst which at excystation produced many new individuals.

In studies on the distribution of testaceans in relation to their habitats, Heal (1961) found *E. acanthophora* in small numbers associated with fen areas, whereas de Graaf (1956) considered this species to have no particular associations.

Euglypha strigosa. This species has been recorded from both wet and dry habitats. de Graaf (1956) noted that in a Netherland fen it preferred drier *Sphagnum*, whilst Heal (1961) described it as a eurytopic species occurring in large numbers in bog hummocks in Northern England and also noted (Heal, 1962) that it appeared to be restricted to the lower levels of the *Sphagnum*. Differences in shell size and shape of specimens of *E. strigosa* were described by Chardez and Leclercq (1963), who suggested that these differences could be related to the type of habitats in which they occur.

Previous work – taxonomy

In a review of the *British Freshwater Rhizopoda* fauna Cash *et al.* (1915) summarize previous taxonomic reports of both *Euglypha acanthophora* and *E. strigosa*. The present account excludes these references and is restricted to subsequent descriptions.

Euglypha acanthophora (Ehrenberg, 1841). This species was originally described as *E. alveolata* but was made a synonym of *E. acanthophora* by Cash *et al.* (1915) who gave a detailed account of their reasons, and this has since been accepted by most authors. They (Cash *et al.* 1915) redescribed *E. acanthophora* and also two varieties which differed mainly in size, and the shape and number of the elongated shell-plates. Further redescriptions and sizes were given by Hoogenraad and de Groot (1940) and Decloitre (1962, 1965), the latter author also described (Decloitre, 1962) seven varieties of *E. acanthophora*.

Euglypha strigosa (Ehrenberg, 1872). Cash *et al.* (1915) described this species as 'the most generally distributed and numerous of the *Euglyphae*', easily distinguished from *E. ciliata* and *E. compressa* by its thickened apertural-plates and circular aperture. In a review of the genus *Euglypha*, Decloitre (1962) redescribed *E. strigosa* and three varieties. Additional sizes and descriptions were given by Bartoš (1963) and Decloitre (1964). The surface ultrastructure of the shell of *E. strigosa* was described by Cambar *et al.* (1964), from metal and carbon-shadowed preparations.

MATERIALS AND METHODS

Euglypha acanthophora was isolated from a sample of sewage sludge from Maple Lodge Works of the Colne Valley Sewage Board, Hertfordshire, in December, 1972.

Euglypha strigosa was isolated from a sample of sphagnum moss collected in the New Forest, near Christchurch, Hampshire, in April, 1970. Both species were obtained from crude cultures made from small portions of each type of material placed in a shallow layer of culture liquid and kept in the laboratory at room temperature, 18–20 °C. Agnotobiotic cultures were kept in small plastic containers, on a thin layer of agar agar (1 per cent in distilled water) with a sterilized wheat grain added prior to setting and covered with a shallow layer of culture liquid. The culture liquid was a 5 per cent (w/v) solution of soil extract, plus 100 mg/l⁻¹ sodium nitrate and 15 mg/l⁻¹ sodium dihydrogen orthophosphate, in distilled water. *E. strigosa* has more recently been kept on 1.5 per cent agar in amoeba saline, with a sterilized rice grain added and covered with a shallow layer of Prescott and James solution (Prescott & James, 1955).

Clonal cultures were established by isolating single active animals. One clone has been used subsequently to produce the working cultures of each species. These clones have been deposited, *E. strigosa* (No. 1520/2), *E. acanthophora* (No. 1520/3), and maintained at the Culture Centre of Algae and Protozoa, The Natural Environment Research Council, Cambridge, England. Subcultures made at 4–5 week intervals are adequate to maintain active animals that readily feed and reproduce.

Optical microscopy. Specimens were examined by both phase-contrast and bright-field illumination, either live or after fixation with Schaudinn's fluid. Smears of fixed animals were stained with either borax carmine or iron haematoxylin.

Scanning-electron microscopy. Live specimens were either cleaned by transferring them from the culture vessels through several changes of triple-distilled water, or fixed in glutaraldehyde and then thoroughly washed. They were then manipulated using a single-hair brush onto a small cover-slip, previously cleaned with acetone and lint-free tissue, to which they adhere when dry. To obtain single siliceous shell-plates, an individual clean specimen was placed on a small segment of cover-slip and covered with a small drop of concentrated sulphuric acid. This was heated gently to evaporate the acid and liberate the plates from the organic cement. These prepared cover-slips were secured to 'Stereoscan' stubs with an electrically conductive paint, 'Silver Dag', prior to being coated evenly with 10–15 nm of gold using the method recommended by Harris *et al.* (1972). The stubs were examined on either the Cambridge Stereoscan Mk II or Stereoscan 600, operating at 15 or 20 kV and the results were recorded on Ilford HP4 film.

DISTRIBUTION

E. acanthophora is usually found in damp mosses, submerged vegetation and standing water, whilst *E. strigosa* is found in similar habitats and additionally in drier mosses and soil.

A full list of locality records and references was given by Decloitre (1962). Since this review several authors, namely, Bartoš (1963), Bonnet (1966), Decloitre (1964, 1965) and Stěpánek (1963), have recorded these species from other localities and these are included here.

The following lists illustrate the geographically widespread distribution of both species :

Euglypha acanthophora

- EUROPE : England, Wales, Scotland, Ireland, France, Holland, Belgium, Germany, Spain, Switzerland, Hungary, Czechoslovakia, Luxembourg, Iceland and Lapland.
 NORTH AMERICA : Greenland, Canada and the United States of America.
 SOUTH AMERICA : Venezuela and Colombia.
 AFRICA : Congo, Cameroons, French West Africa, South Africa and Madagascar.
 ASIA : China, Japan, Java and Sumatra.
 AUSTRALASIA : Australia and New Zealand.

Euglypha strigosa

- EUROPE : England, Wales, Scotland, Ireland, France, Belgium, Holland, Spain, Germany, Switzerland, Italy, Hungary, Czechoslovakia, Bulgaria, Rumania, Spitzbergen, Finland and Iceland.
 NORTH AMERICA : Greenland, Canada and the United States of America.
 SOUTH AMERICA : Central America, Colombia, Venezuela and Chile.
 AFRICA : South Africa, French West Africa, Madagascar and the Canary Islands.
 ASIA : Krakatoa Island, Borneo and Java.
 AUSTRALASIA : Australia, Tasmania and New Zealand.
 ANTARCTICA : Adelaide Island.

MORPHOLOGY AND VARIATION

Euglypha acanthophora. The shell has a mean length of $70.8 \mu\text{m} \pm 9.6 \mu\text{m}$ and a mean breadth of $39.0 \mu\text{m} \pm 4.4 \mu\text{m}$; it is elliptical in shape through the major axis and circular through the minor axis (Pl. 1, fig. A), and is made up of three different types of siliceous plates – apertural-plates (Pl. 1, fig. E), shell-plates (Pl. 1, fig. B) and elongated shell-plates (Pl. 1, fig. B). The aperture is terminal, circular and has a mean diameter of $18.4 \mu\text{m} \pm 4.9 \mu\text{m}$, it is surrounded by between 10 and 13 evenly spaced apertural-plates (Pl. 1, fig. D). Each apertural-plate is roughly circular in shape and varies in size from $8.1 \mu\text{m}$ to $12.5 \mu\text{m}$ in length and $7.2 \mu\text{m}$ to $11.4 \mu\text{m}$ in width. The dentate edge of the apertural-plate is slightly thickened and carries a median tooth with either four or five smaller lateral teeth on each side (Pl. 1, figs. C and E). The apertural-plates are found not only bordering the aperture, but are also seen in the second and third row inside the aperture (Pl. 1, fig. E). There are approximately 200 shell-plates which range in size from $10.9 \mu\text{m}$ to $12 \mu\text{m}$ in length and $7.9 \mu\text{m}$ to $9.1 \mu\text{m}$ in width. They are roughly circular in shape with the anterior edge usually having three small pointed projections (Fig. 1a), and are arranged regularly in alternate longitudinal rows (Pl. 1, fig. A). In the posterior

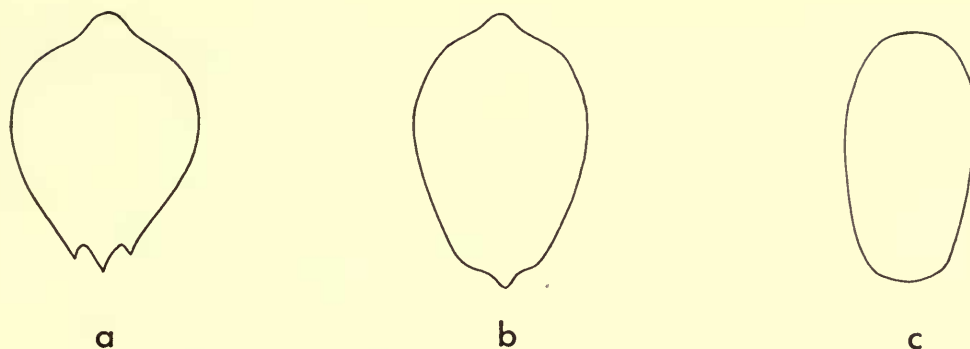


FIG. 1. Diagram to show the comparative shape of shell-plates (a) *E. acanthophora* from anterior region, (b) *E. acanthophora* from posterior region, (c) *E. strigosa*.

region of the shell there are normally between one to six elongated shell-plates which have one edge tapering to a fine point (Pl. 1, fig. B). These plates are approximately twice as long as a normal shell-plate, nevertheless, the projections break easily so that they are often seen to be short and end bluntly. The pointed edge of these elongated shell-plates is usually directed posteriorly so that they are seen to project from the outline of the shell, but on occasions they follow the curvature of the shell and are difficult to see by light microscopy.

Abnormally shaped shells occur occasionally, although the incidence of such forms appears to be less than three per cent. They are usually specimens with either deformed anterior regions or small specimens, about half or two-thirds normal length, with wide apertures. The first type is the most frequent abnormal shell form, having a normal arrangement of shell-plates in the posterior region but anterior to the middle region the shell-plates are compacted together haphazardly, so that the shell only attains half the normal length. These deformed shells usually collapse when air-dried whilst normal shells retain their shape. The animals inside these deformed shells appear to possess the normal cytoplasmic organelles.

Euglypha strigosa. The shell has a mean length of $81.3 \mu\text{m} \pm 3.8 \mu\text{m}$ and a mean breadth of $42.2 \mu\text{m} \pm 6 \mu\text{m}$; it is elliptical in shape through both the major and minor axes (Pl. 2, fig. A). Two distinct types of siliceous plates make up the shell, apertural-plates and shell-plates, and in addition there are numerous elongated siliceous spines (Pl. 2, fig. A). The aperture is terminal and circular, with a mean diameter of $14.9 \mu\text{m} \pm 0.8 \mu\text{m}$; it is surrounded by between 10 and 13 evenly spaced, apertural-plates – the usual number of apertural-plates is 11 (Pl. 2, fig. E). Each apertural-plate is roughly circular in shape and varies in size from $9.2 \mu\text{m}$ to $10.8 \mu\text{m}$ in length and $7.8 \mu\text{m}$ to $8.5 \mu\text{m}$ in width. One margin is distinctly thickened and carries a large median tooth with either three or four smaller teeth on each side (Pl. 2, fig. C). The shell-plates are roughly oval in shape (Fig. 1c), with a size range of from $8.0 \mu\text{m}$ to $11.3 \mu\text{m}$ in length and $4.1 \mu\text{m}$ to $6.4 \mu\text{m}$ in width, and they are arranged in regularly spaced, alternate longitudinal rows. The thin, elongated spines vary in shape and size from small pear-shaped spines approximately $2 \mu\text{m}$ long to thin

spines 23 μm long, and they project from any part of the shell surface (Pl. 1, fig. A). They are found at the junction of shell-plates, where they are held in position by a similar organic cement material to that which holds the shell-plates together in *E. rotunda* (Hedley and Ogden, 1973). This has been confirmed from the present authors unpublished observations of the fine structure of *E. strigosa*. The spines are single structures, although two or more are often seen projecting from the same junction as though they were connected. They usually project from the surface at right-angles, but they may lie close to the surface and point in any direction. There appears to be no regular arrangement in the distribution of the spines, although usually only the smaller spines are seen at the junction of the apertural-plate and shell-plate region, and the largest spines seem to lie along the lateral margins. The spines appear to be removed easily by abrasive action (Pl. 2, fig. B).

No abnormal forms of this species have been seen in our cultures.

REPRODUCTION

Euglypha acanthophora. Growth curves produced from observations made on three replicate cultures gave an estimated doubling time of between 2.3 and 2.8 days. Cytoplasmic division proceeds in a similar sequence to that previously described for *E. rotunda* by Hedley and Ogden (1973). The approximate time taken to produce the daughter shell is 25 minutes, whereas the time taken for the completion of binary fission is two hours. The animals are usually active after division with strong pseudopodial movement (Fig. 2).

Eight specimens with abnormal apertures were isolated and after six days had divided to produce 27 normal individuals. Observations on individual, dividing abnormal forms, such as those with a wide aperture and others being half normal size, show that they often produced smaller individuals with apparently the normal shell shape. One abnormal animal at division produced an almost normal shell, a small shell and some loose shell-plates (Fig. 3). Cytoplasm was present in the larger daughter shell, and the group did not separate after the normal time taken for division, but moved after this time as a group. During division in a normal individual, at the time when all the reserve shell-plates had passed into the pseudopodial trunk, a small circle of shell-plates became detached from the posterior end of the daughter shell. Binary fission proceeded normally and the two individuals separated, leaving behind the circle of shell-plates.

Euglypha strigosa. An estimated doubling time of between 3.9 and 4.3 days was obtained from growth curves produced from observations on three replicate cultures. Division is preceded by the retraction of the filose pseudopodia, followed by the extrusion of a large pseudopodial trunk and shortly after by the apertural-plates (Pl. 2, fig. D). The shell-plates are then arranged progressively until the daughter-shell is almost complete, at which point the spines are pushed out between the shell-plates with considerable movement. This continues for a short time after the shells are equal in size, but then the movement of both shell-plates and spines ceases abruptly. The time taken to produce a daughter-shell is approximately 50 minutes, with complete binary fission taking about three hours.

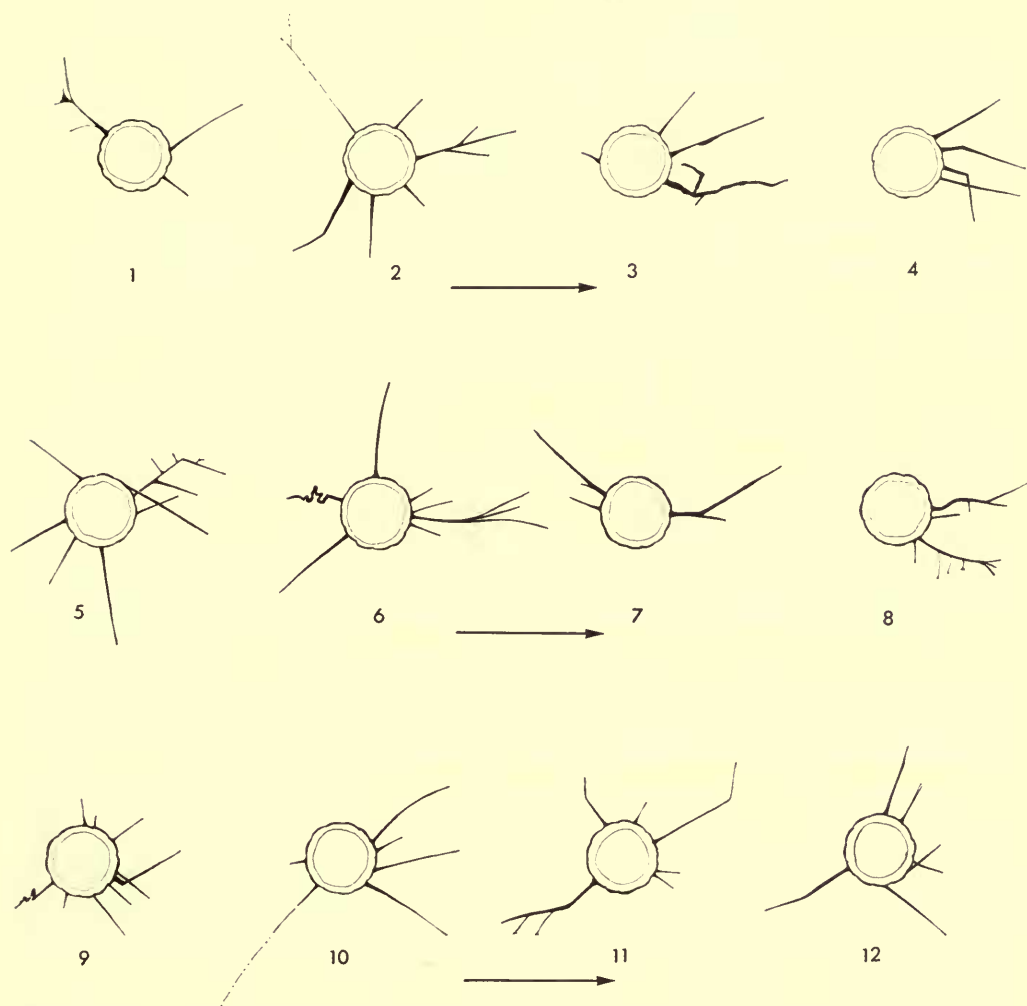


FIG. 2. Drawings of a moving specimen of *E. acanthophora* observed over a period of 30 minutes, to show the diversity of pseudopodial movement; the arrow denotes the direction of movement.

It was noted that in preparations of dividing specimens for scanning electron microscopy, the daughter-shell consistently collapsed. This is possibly due to an incomplete stabilization of the organic cement lining.

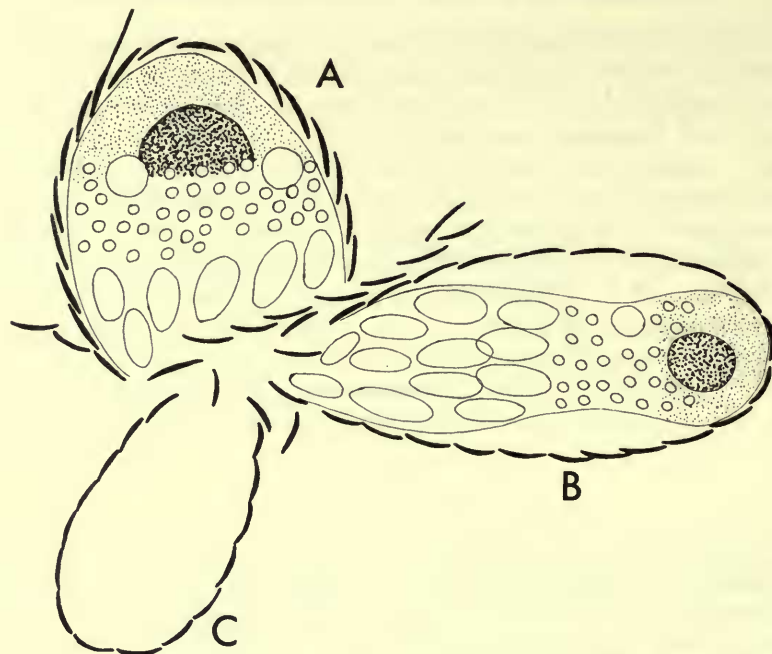


FIG. 3. Diagram representing the division products of an abnormal form ; A – abnormal form, B – apparently normal daughter and C – a small form without cytoplasm.

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Dr R. H. HEDLEY
C. G. OGDEN
JUNE I. KRAFFT
BRITISH MUSEUM (NATURAL HISTORY)
CROMWELL ROAD
LONDON SW7 5BD

PLATE I

- A. Lateral view of *E. acanthophora* showing the arrangement of the shell-plates. × 1500
- B. Preparation of shell-plates to illustrate two elongated shell-plates. × 3000
- C. Lateral view of single apertural-plate with characteristic teeth. × 7600
- D. Aperture of *E. acanthophora* showing the arrangement of the apertural-plates. × 2900
- E. Ventral view of apertural-plate preparation, to show thickened dentate edge and the arrangement of three rows of aperture-plates. × 3500

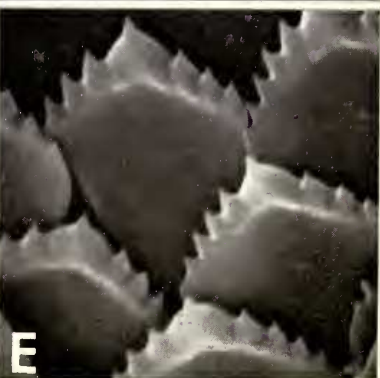


PLATE 2

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|----|---|--------|
| A. | Lateral view of <i>E. strigosa</i> to show the distribution of siliceous spines. | × 1200 |
| B. | Area of shell surface illustrating the holes left by removal of spines. | × 5000 |
| C. | Lateral view of a single apertural-plate. | × 4700 |
| D. | Apertural view of the first stage of division ; note the large pseudopodial trunk (arrowed) with some daughter-shell apertural-plates arranged on it. | × 2700 |
| E. | Aperture of <i>E. strigosa</i> with eleven evenly spaced apertural-plates. | × 3000 |

